THE FUNCTIONAL PROPERTIES OF CHEMICALLY AND ENZYMATICALLY MODIFIED WHEAT GLUTEN

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ABSTRACT
This research was carried out to investigate the effect of chemically (using L-Cysteine) and enzymatically (by papain) modification on the functional properties of hard wheat gluten. The modification process is run by both methods individually and synergistically. The results showed an increase in the degree of hydrolysis (DH) of lyophilized wheat gluten upon adding the reducing agent at different concentrations (50, 100, 150, 200, 250 ppm). The values for (DH) were (5.29, 6.18, 6.22, 6.26, and 7.48%) respectively. While for papain (2000 units / g protein) those values were (10.25, 12.71, 13.54, 13.88, 15.62%) respectively when the reducing agent (same concentration above) and papain were used synergistically. The functional properties (water holding capacity, emulsifying ability and stability, foam formation and stability, protein solubility, and oil holding capacity) of the gluten under study were examined. The results showed that the enzymatic modification significantly improved the functional properties of the experimental proteins compared to the reducing agent modification.

Key word: antioxidant properties, pepsin, trypsin, papain, gluten hydrolysates.
Part of M. Sc. thesis of the 1st author.

الخصائص الوظيفية لغلوتين القمح المحور كيميائيا وإنزيميا

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المستخلص
أجريت الدراسة الحالية لغرض التعرف على تأثير التحوير الكيميائي (باستعمال L– Cysteine) والتحوير الإنزيمي (بأنزيم البابين) لكلوتين الحنطة الصلبة على خصائصها الوظيفية. أجريت عملية التحوير بالطريقتين بصورة منفردة وبصورة تآزرية. أشارت النتائج إلى ازدياد درجة تحلل كلوتين الحنطة المجفد عند إضافة العامل المختزل لوحده بتراكيز مختلفة و كانت درجة التحلل بواقع (5.29، 6.18، 6.22، 6.26، 7.48 %) عند التراكيز (50، 100، 150، 200، 250 جزء بالمليون) على التوالي. بينما كانت درجة التحلل بواقع (10.25 ، 12.71 ، 13.54 ، 13.88، 15.62 %) على التوالي، عند استخدام العامل المختزل بوجود إنزيم البابين بتركيز (2000 وحدة / غم بروتين) و تراكيز العامل المختزل ذاتها المشار أليها أعلاه، مما يدلل على الدور ألتآزري للعاملين الكيميائي والإنزيمي. تم فحص الخصائص الوظيفية (قدرة حمل بالماء، قدرة الاستحلاب والثبات، تكوين الرغوة وثباتها، قابلية ذوبان البروتين، وقدرة حمل الزيت) للبروتين قيد الدراسة. أظهرت النتائج أن التعديل الأنزيمي أدى إلى تحسين الخواص الوظيفية للبروتينات المختارة معنوياً مقارنةً مع التعديل بالعامل المختزل.

الكلمات المفتاحية: الخصائص المضادة للأكسدة، ببسين، تربسين، بابين، متحللات الكلوتين
- جزء من رسالة الماجستير للباحث الأول.

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INTRODUCTION

Wheat is one of the common generally grown cereal crops in the world and it has been used mainly in the flour and bread making. Some types of wheat (such as *Triticum aestivum*) are suitable for making bread, while others (such as Triticum durum) are proper for making pasta and noodles. The yearly global wheat production is around 600 million tons (13). Wheat contains many proteins, glutenin soluble in alkali and diluted acid solutions, gliadin soluble in ethyl alcohol (70%), albumin proteins dissolvable in water, and globulin proteins dissolvable in dilute saline solutions. The foremost factor in the fitness of wheat varieties for making various types of bread products is the ability to form a gluten network. Gluten is known as the main element of wheat proteins, which gives the flexibility and strength of the dough, and it is the elastic mass that remains when washing wheat dough to remove starch particles and water-soluble ingredients (13). Besides, gluten is a mix of prolamin proteins, which are found principally in wheat, barley, corn, and oats. (3). Wheat gluten is the primary measure of the rheological characteristics of wheat dough and the consistency of wheat bakery products that adopt direct kneading means (27). The properties of elasticity and viscosity are related to the quality and quantity of gluten, which influences the volume, texture, form, and taste of baking products (26). The original wheat protein can be modified to improve its property and nutritional value (7). The enzymatic alteration considered the safest method to get an excellent functional and nutritional characteristic (24). Therefore several enzymes such as pepsin, trypsin, and papain has been used to improve the solubility of wheat gluten, (18). This study was designed to investigate the functional properties of durum wheat gluten before and after modification using reducing agent (L-Cysteine) alone and synergistic modification using an enzyme (papain) and the L-Cysteine.

MATERIALS AND METHODS.

Wheat samples.

The durum wheat (*Triticum durum*) used in 1this study was native (*Smeto*) variety, grown at Mosul region in 2018. Wheat grains were conditioned to 14 % moisture before milling.

Enzymes; Papain was used ,(BDH, England product). **Preparation of a reducing agent (L-Cysteine);** A certain amount of L-Cysteine (0.025) g was dissolved in 10 ml of distilled water, then (1, 0.8, 0.6, 0.4, 0.2 ml) of this solution were added to the dough ingredients , to achieve (250, 200, 150, 100, 50) ppm of the reducing agent respectively.

Preparation of modified gluten using the reducing agent (L-Cysteine); The method of Kumar *et al.,* (14) was followed with some modifications. Ten grams of durum wheat flour (*Smeto*) was kneaded with brine (2% NaCl). Then the reducing agent (L-Cysteine) was added at different concentrations, and the dough was immersed in brine for 45 minutes. The dough was gently washed under tap water to get the gluten which then was lyophilized at (-75)°C.

Enzymatic modification of wheat gluten using Papain enzyme ; Gluten hydrolysates were prepared using papain, according to (4) with some modifications. The gluten was mixed with distilled water in a ratio of 1:20, and the pH was adjusted to 10 with NaOH (0.1M) and incubated at 50˚C for 1 hour until the gluten completely dissolved. The pH of the obtained solution re-adjusted to 8 using hydrochloric acid (0.1M) and incubated for 15 minutes at 37˚C. The enzyme was added at a concentration of 2000 units per 1 gram of gluten and incubated at 50˚ C for 4hr. An aliquot of the hydrolysates was taken after (1,2,3,4 hr.), then the reaction terminated by placing the samples in a boiling water bath for 5 minutes, and centrifuging at 5000xg for 15 minutes. The supernatant collected and stored at (-18 ˚C) until use.

Chemical analysis; Proximate compositions of wheat and flours were determined using AOAC methods (3). Total carbohydrate was calculated by difference

Determination of the Degree of Hydrolysis (DH); The degree of hydrolysis was tested, according to (16). The standard solution of L-Lucien (55Mm) was prepared by dissolving 0.361g L-Lucien in a small amount of distilled water, and the volume was completed to 50 ml. The required concentrations were prepared, as shown in Table 1.

Procedure ; To 0.250 ml of each of the above solutions, 2 ml of SDS(1%) and 2 ml sodium

phosphate (0.2125 M) at pH 8.2 and 2ml of TNBS solution (0.1%) were added. The mixture was incubated at 50˚C for 1hour at dark place. The reaction was stopped by adding 4 ml of HCl solution (1M). The samples were kept at room temperature for 30 minutes, and the absorbency was read at 340 nm. The standard curve was plotted as the relation between the concentration of the L-Lucien and the absorbance reading at 340 nm.

Table 1. L-Lucien concentrations used in standard curve for the degree of hydrolysis determination

Concentrations (mM)	Stock solution(ul)	D . W (ul)	Final Volume (ul)
0	0	1000	1000
5	50	950	1000
15	150	850	1000
25	250	750	1000
35	350	650	1000
45	450	550	1000
55	550	450	1000

The studied samples (0.250 ml of each) were transferred to a test tube and subjected to the above steps. NH_3 groups were calculated using the standard Lucien amino acid curve and the degree of degradation was calculated according to the following equation (12):

$DH = [(L_t – L_0) / (L_{max} – L_0)] * 100$

L_t = concentration of α-NH_3 released in the time L_0 =α-NH_3 found in the original protein sample . L_{max}= total α- NH3 in the undigested sample , which can be obtained after acidification using HCL (6 M) at 120˚C for 24h

Determination of antioxidant activity

DPPH Radical-Scavenging Activity (RSA)

The RSA was measured according to (15) with some modulations. One ml of the sample (4 mg/ml) was mixed with 1 ml of DPPH solution (0.1 M). The mixture kept at the dark place at room temperature for 30 minutes and then centrifuged at 10,000x g for 5 min. The absorbency was measured at 517 nm, and the percentage of the scavenging activity was calculated according to the following equation Radical Scavenging activity A = [C – (B–A)/ C] x100

A= Spectrophotometer reading of the tested sample at517 nm wavelength.

B = the absorbance reading of the control sample at 517 nm (prepared by mixing 1 ml of ethyl alcohol with 1 ml of the sample under study).

C = reading of the positive control at 517 nm (obtained from mixing 1 ml of DPPH with 1 ml of distilled water).

Functional properties

Solubility determination: Solubility of the protein was determined according to the method suggested by (6) . A sample of gluten (50 mg) was dissolved in 20 ml of distilled water and the pH adjusted to different values (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12), and left for 15 minutes under controlled pH, then centrifuged at 10000 * g for 15 minutes. The supernatant was collected and the total nitrogen content was estimated . The percentages of solubility were calculated as follows : Solubility % = protein content in the supernatant/ protein content in the sample *x* 100 .

Water holding capacity determination

Onsaard *et al.* (19) method was followed with some modification, 0.5 g of the experimental sample was mixed with 10ml distilled water, vortexed for 5 minutes . The pH was adjusted to (4, 7, 12) and left at room temperature for 15 minutes, then centrifuged at 10,000 g for 10 minutes . Water holding capacity was calculated using the following equation.

W.H.C = W2-W1/ W0

W2 = tube weight + weight of the precipitate after water removal

W1 = tube weight + Sample before water addition

W0 = weight of Sample .

Oil holding capacity determination

Onsaard *et al*. (19) method was followed with some modification, 0.5 g of the sample was mixed with 10ml sun flower oil placed on the vortex for 15 minutes , then the pH adjusted to (4, 7, 12) and left at room temperature for 15 minutes , then centrifuged at 10,000 g for 10 minutes . Oil binding Capacity was calculated by the following equation

Oil Binding capacity (gm oil /gm sample) = F2-F1 / F0

F0 = weight of the sample .
F1 = tube weight + sample weight before adding oil
F2= weight of the tube + weight of the deposit after removing the oil .

Estimation of foam formation capacity and stability: Cano – Medina *et al.*(5) method was adopted with some modification. One gram of the experimental samples was mixed with small amount of distilled water for one minute, and the volume completed to 100 ml , the pH of the obtained mixtures were adjusted to (4 , 7 , 12), 50 ml of each sample were placed in 150 ml flasks ,then mixed for one minute at high speed and then transferred to a 100 ml graduated cylinder . The volume was measured before and after whipping. The ratio of foam capacity and stability was calculated as follow:

Foam capacity % = volume after whipping – volume before whipping / volume before whipping *100

Foam stability % = foam volume after a certain time/foam volume at time zero *x* 100

Emulsification and emulsion stability

Sharm *et al.*,(25) method was adopted with some modification, 5 ml of the modified gluten samples (1%) at three different pH values (4, 7, 12) were mixed with 5 ml sunflower oil. The mixture was homogenized, (10,000 cycles / minute), Centrifuged at (3500*g) for 5 minutes and the emulsions layer volume was measured by `graduated cylinder. The percentage of emulsification capacity was calculated using the following equation:

Emulsification capacity % = Emulsion layer volume/ Total volume * 100

The stability of emulsion was measured by placing the emulsion in a water bath at 85 C for 30 min and then centrifugation (3500*g) for (5) minute and the emulsion layer volume was measured . Emulsion stability was calculated using the following equation

Stability of emulsion = Emulsion layer after heating / Total volume before heating *100

Statistical analysis; Statistical Analysis System (SAS) (22) was used for the analysis of data, to study the effect of different treatments in the studied traits in full randomized design (CRD). The differences between mean were compared with the least significant difference (LSD).

RESULTS AND DISCUSSION

Chemical components of durum wheat, wheat flour, dried and Lyophilized durum wheat gluten and commercial gluten

Data in Table 2 shows the percentages of moisture, protein, fat, ash, fiber and carbohydrates of durum wheat , wheat flour(72-76% extracted) and lyophilized durum wheat gluten. The percentages of moisture were (6.61, 9.6, 4.49 %) respectively. Moisture content has a significant impact on the quality of wheat storage and is also an essential factor in evaluating the quality of the resulting flour and its water absorption. It has been noticed, due to the wheat conditioning, moisture percentage has increased in the flour. The protein content of wheat and flour were 17.5% and 13%, respectively. Protein is of great importance in determining product quality. The same table also showed that the fat percentage were (2.64, 1.91%) respectively. Many studies confirm the importance of flour fat in the manufacture of bread and the rheological properties of the dough despite its small quantity. The percentage of ash was 3.07 and 1.9 %, respectively. Ash content is an important measure related to the quality of the milling process, and also it is a strong indicator of flour color and purity. It is noted that the ratio of fibers does not correspond to the percentages range indicated by (33), who pointed out that the percentage of fiber in Iraqi wheat varieties ranged between 2 - 2.7%. Iuliana *et al.,* (11) reported that the percentage of carbohydrates for wheat varieties ranged from 65-75%, and this is similar to our finding in the Iraqi wheat strain in this study which was 67.07%..

Table 2.Chemical composition of durum wheat and wheat flour(72%) and lyophilized durum wheat gluten (%).

Treatment	Ash	Carbohydrate	Fiber	Protein	Fat	Moisture
Durum Wheat	3.07	67.07	3.11	17.5	2.64	6.61
Flour 72-76%	1.9	71.49	2.1	13	1.91	9.6
lyophilized gluten	2.90	10.04	0.21	80.5	1.86	4.49

Results in Table 2 show the chemical composition of lyophilized durum wheat gluten. The percentage of moisture, protein, fat, fiber, ash and carbohydrates for the obtained gluten were (4.49, 80.50, 1.86, 0.21, 2.90, 10.04%), respectively. The obtained values differed with (2; 29). The difference in the chemical composition of the extracted gluten may be due to differences in gluten sources, extraction method, and sample drying methods.

Degree of hydrolysis for wheat gluten hydrolysates using the reducing agent and papain enzyme: Figure 1. shows the degrees of hydrolysis (DH%) of lyophilized wheat gluten treated with a reducing agent (L-Cysteine) at concentrations of (50, 100, 150, 200, 250 ppm) and papain at a concentration of (2000 units/g of gluten) at pH 8 and temperature of 50 ° C for 4 hours. The results revealed that the DH% were positively proportional to the concentration of the added reducing agent (L-Cysteine) individually and/or with 2000 enzyme unit of papain. The highest value for DH% was (15.62%) for gluten treated with the papain in the presence of the reducing agent (at a concentration 250 ppm). While the DH% by the reducing agent alone at the same concentration (250ppm) was(7.48%). The lowest DH% was recorded when (50ppm) of reducing agent used (0.25 %) , in the absence of the papain enzyme. The results also indicated that DH% was increased as the concentration of the reducing agent increased in the presence of the enzyme, which indicates the synergistic role of the chemical and enzymatic modification.

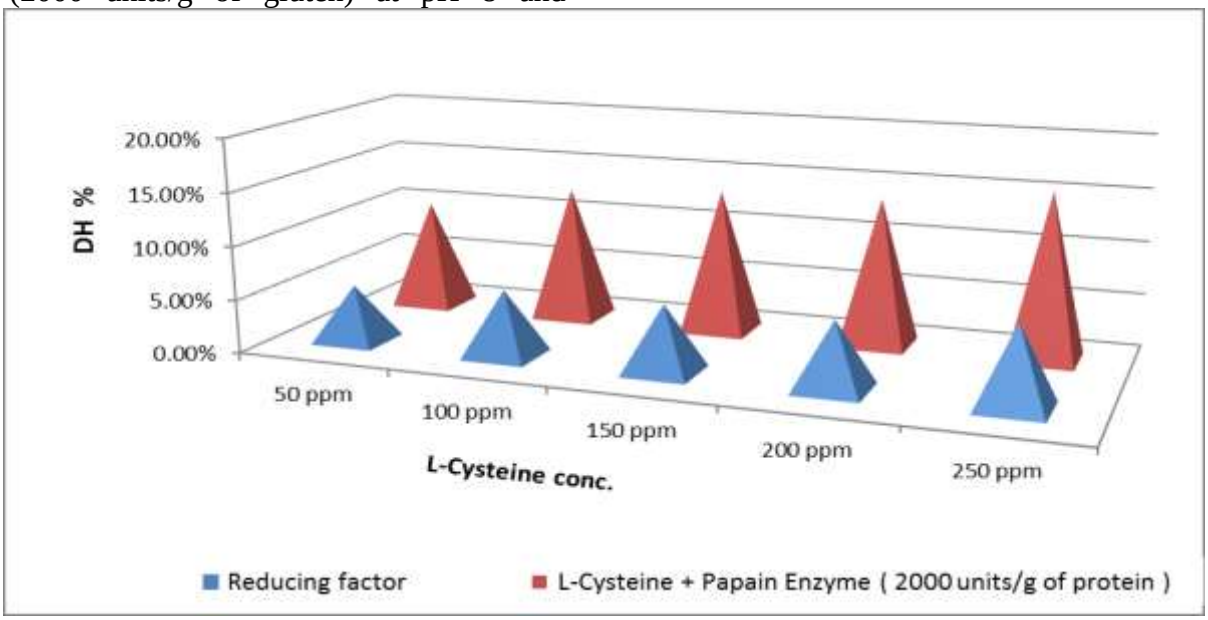


Figure 1. Degrees of hydrolysis (% DH) for gluten treated with reducing agent different conc. (50, 100, 150, 200, 250 ppm) and papain enzyme at a concentration of (2000 units / g of protein) at a pH of 8 and a temperature of 50 ° C for 4 hours

Kumar, (14) stated that the rate of wet and dry gluten extraction from two type of hard wheat due enzyme treatment and the reducing agents (proteases, glutathione, L-Cysteine) adding was decreased, as well as the strength of the dough in these treatments. This decrease was concentration dependent with all factors. This was attributed to the fact that the reducing agents cause the breakdown of the covalent sulfur bonds between and within the protein chains, which in turn leads to a decrease in the molecular weights of these proteins as a result of the action of these factors, and these effects increased when those factors were added together. The results of this study were different than that of (34) who found in studying the synergistic role between Cysteine and alcalase that the DH after an hour was (70%) and (43%) with the presence and absence of Cysteine, respectively, the reason for this may be due to the addition of the reducing agent after adding the enzyme, unlike what was done in this study. Phongthai *et al.* (20)found that the synergistic effect of pepsin and trypsin on a rice husk protein extract resulted in the production of bioactive, low-molecular-weight peptides

Measurement of antioxidant activity. Radical-Scavenging Activity (RSA)

Figure 2. shows the RSA of wheat gluten treated with L-Cysteine and papain individually and synergistically at pH (8) and temperature 50° C for (4) hours, assessed by using the DPPH assay. The results revealed that the radical-scavenging ability of the gluten hydrolysates increased as the reducing agent concentration increased and it reached the highest value when (250 ppm) was used. When the dried gluten treated with L-Cysteine and papain, the RSA reached (27.41, 38.14,%), respectively. Results in figures 1 and 2, reveal that there were a direct relationship between the DH of wheat gluten and the free radicals Scavenging activity. The results of this study agreed with that reported by Iuliana *et al.* (11), who found that the ability of decomposers to inhibit free radicals increases with the progression of the degree of degradation of gluten using alcalase. This enzyme is known to be one of the internal enzymes types that work on the analysis of internal peptide bonds, producing decomposers with a high radical scavenging ability. The same researcher also found that the pre-thermal treatment of gluten and then exposing it to the enzymatic action led to an increase in the D.H. Thus the antioxidant activity increased as a result of this process. Yan *et al.*(32) reported similar results , where they found that the antioxidant activity of the rice residue degraded by several enzymes (Papain, Flavourzyme, Protomex) singly and in compaination was in an inverse relationship with their molecular weights. The ability to suppress free radicals for all enzymatic decomposers increased with the progression of the degradation time and the increase in the degree of degradation for each treatment separately or synergistically. This is due to the role of the enzymatic activity in increasing the number of amino acids exposed to the medium with the progress of the degradation process, which in turn plays a positive role in increasing the antioxidant activity

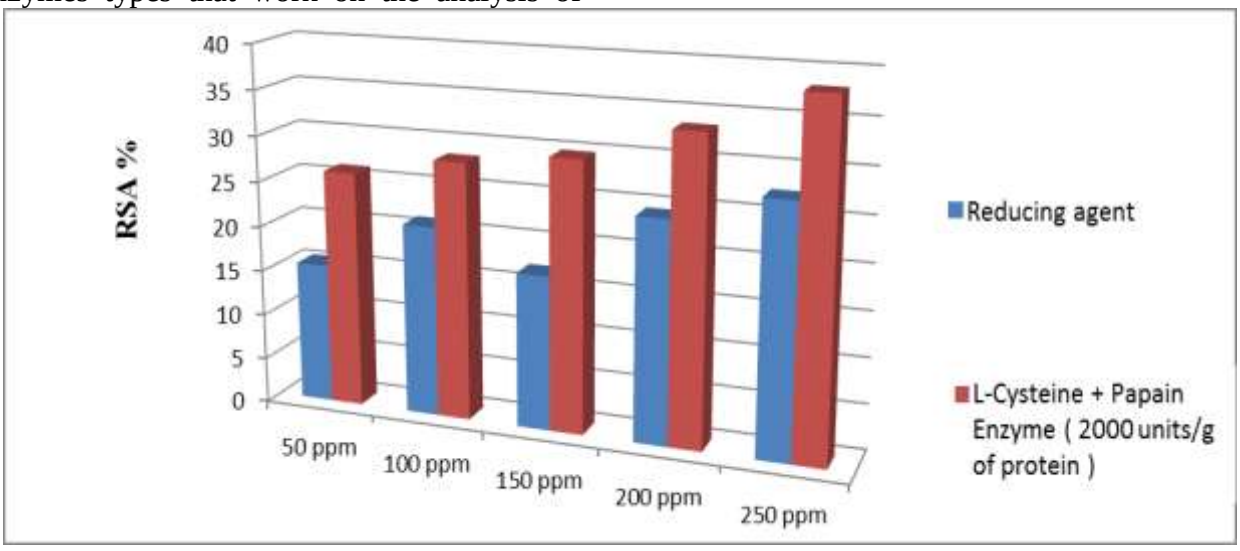
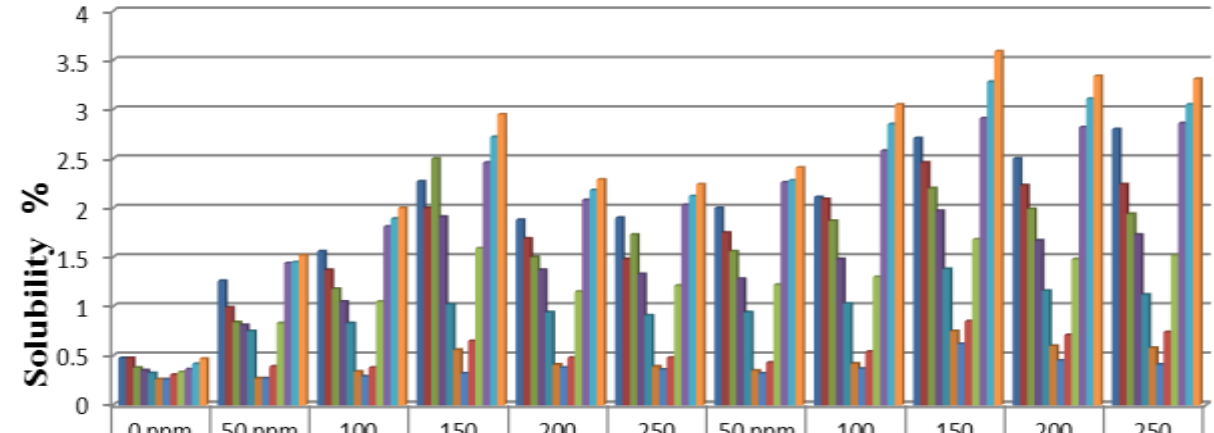


Figure 2.The radical scavenging ability of lyophilized wheat gluten treated with the reducing agent L-Cysteine (different conc.) and papain enzyme at a concentration of (2000 units / g of protein) at a pH of (8) and a temperature of (50) ° C for (4) hours, assessed by using the DPPH assay

Functional properties.

Solubility: Figure 3 shows the solubility of wheat gluten treated with the reducing agent (L-Cysteine) and the reducing agent with papain enzyme at a concentration (2000 units / g of protein) synergistically at different pH values for 4 hours. It has been noticed that treating of wheat gluten with the reducing agent alone led to an improvement in the solubility of gluten at all concentrations and all pH values, where the highest values were at the concentration of (150ppm) and the pH of (12), while the least solubility was in the concentration of (50ppm) at pH (1). The results also indicate that treating the lyophilized gluten from hard wheat with the reducing agent and papain enzyme synergistically improved the solubility of gluten at all concentrations and all pH values more than that treated with L-Cysteine alone.

The highest values were the noticed when (150ppm) of reducing agent was used at pH (12), and the lowest solubility was at the concentration of (50ppm) at the pH (1). The highest solubility values in the presence of the enzyme were (3.59, 3.28, 2.91, 1.68, 0.85, 0.62, 0.75, 1.38, 1.97, 2.2, 2.46, 2.71%) at pH values (12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1) respectively. Cysteine is a reducing agent that breaks down sulfur bonds (S-S) in gluten leading to an increase in the number of peptides of low molecular weight. Cysteine affects the components of gluten (gliadin and gluteinin), which facilitates the enzyme action, which may increase the solubility of gluten further. Mejri et al., (18) studied the effect of Cysteine as a reducing agent on the solubility of gluten at pH values ranged from 2-12, where the minimum solubility of native gluten was reached (33%) at pH 6, while the solubility increased at the other pH levels. In the presence and absence of Cysteine. The treated gluten recorded the highest solubility as compared to crude gluten at all pH values, and the lowest solubility of the treated gluten reached (55%) at pH (7). The pH (6-7) recorded the lowest solubility because it represents the pH values which are adjacent to the isoelectric point. In contrast, the increase in solubility in other alkali and acidic pH values can be attributed to an increase in the charges carried by the protein (23). Cysteine reduce the (S-S) bonds , which leads to a weakening of the protein structure and a decrease in the hydrophobic surfaces. Therefore, protein solubility will be improved.



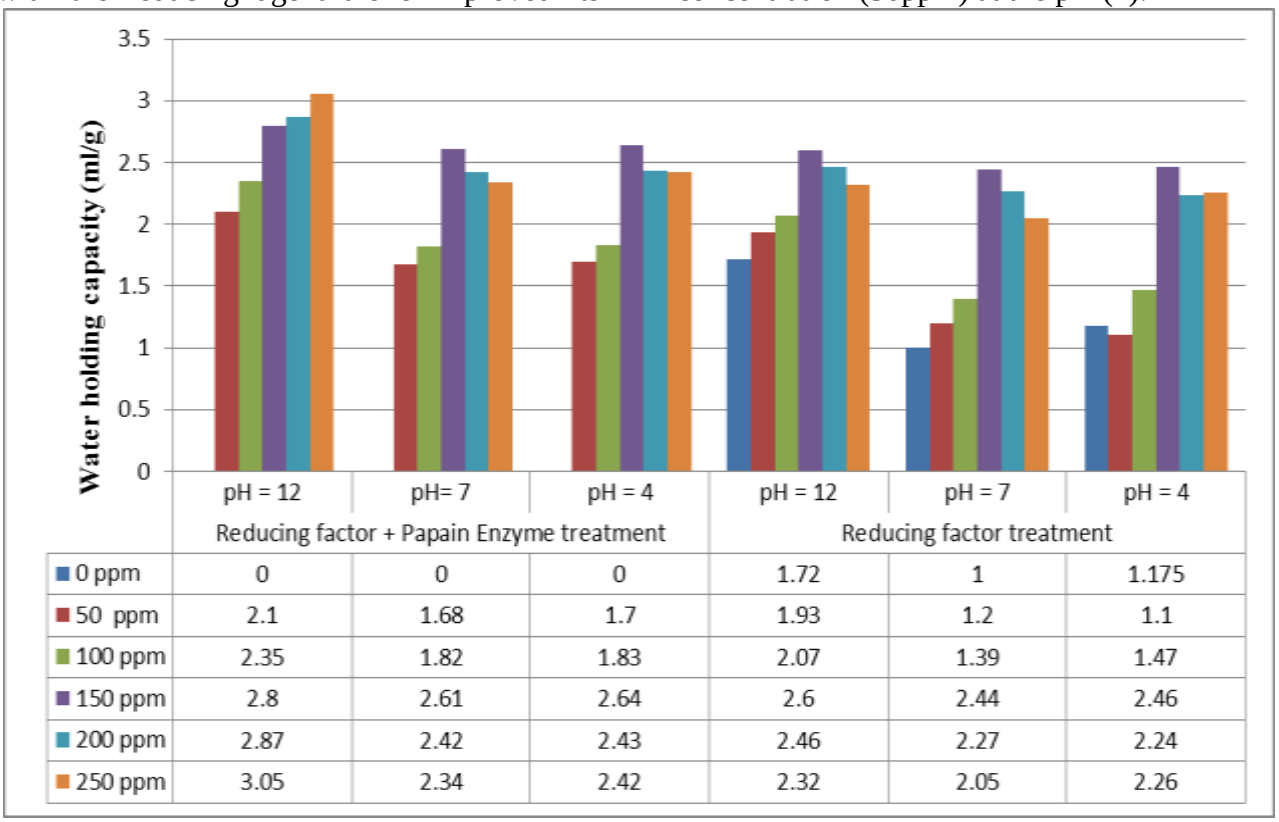
	0 ppm	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm
	Reducing factor treatment						Reducing factor + Papain Enzyme treatment				
PH= 1	0.477	1.26	1.56	2.27	1.88	1.9	2	2.11	2.71	2.5	2.8
PH= 2	0.477	0.99	1.37	2	1.69	1.48	1.75	2.09	2.46	2.23	2.24
PH= 3	0.379	0.84	1.18	2.5	1.5	1.73	1.56	1.87	2.2	1.99	1.94
PH= 4	0.351	0.81	1.05	1.91	1.37	1.33	1.28	1.48	1.97	1.67	1.73
PH= 5	0.324	0.75	0.83	1.02	0.94	0.91	0.94	1.03	1.38	1.16	1.12
PH= 6	0.261	0.27	0.34	0.56	0.41	0.39	0.35	0.42	0.75	0.6	0.58
PH= 7	0.26	0.27	0.29	0.32	0.38	0.36	0.32	0.37	0.62	0.45	0.41
PH= 8	0.306	0.39	0.38	0.65	0.48	0.48	0.43	0.54	0.85	0.71	0.74
PH= 9	0.333	0.83	1.05	1.59	1.15	1.21	1.22	1.3	1.68	1.48	1.52
PH= 10	0.362	1.44	1.81	2.46	2.08	2.03	2.26	2.58	2.91	2.82	2.86
PH= 11	0.416	1.45	1.89	2.72	2.18	2.12	2.28	2.85	3.28	3.11	3.05
PH= 12	0.468	1.52	2	2.95	2.29	2.24	2.41	3.05	3.59	3.34	3.31

Figure 3. The solubility of wheat gluten treated with the reducing agent (L-Cysteine) at different concentrations and with papain at a concentration of (2000 units / g of protein) at different pH values and a temperature of (50C) for (4) hours

The solubility of plant proteins decreases due to the high percentage of hydrophobic amino acids in their composition, unlike animal proteins, their solubility is very low in the

water due to the high ratio of hydrophobic amino acids to hydrophilic in their composition (30).

Water holding capacity.Figure 4. shows the water holding capacity for wheat gluten treated with the reducing agent (L-Cysteine) alone and reducing agent with papain enzyme at a concentration (2000 units / g of protein) synergistically at pH values (12,7,4) for (4) hours. The results indicate that treating gluten with the reducing agent alone improved its ability to retain water at all pH values as the highest values were at a concentration (150 ppm) at pH (12). The lowest value were at the concentration (50ppm) and pH (4). The results also indicate that synergistic treatment of gluten with reducing agent and papain enzyme improved its water holding capacity at all pH values more than that with L-Cysteine alone, as the values were highest at (250 ppm) and at pH (12). The lowest values were in the concentration (50ppm) at the pH (1).



	pH = 12	pH= 7	pH = 4	pH = 12	pH = 7	pH = 4
0 ppm	0	0	0	1.72	1	1.175
50 ppm	2.1	1.68	1.7	1.93	1.2	1.1
100 ppm	2.35	1.82	1.83	2.07	1.39	1.47
150 ppm	2.8	2.61	2.64	2.6	2.44	2.46
200 ppm	2.87	2.42	2.43	2.46	2.27	2.24
250 ppm	3.05	2.34	2.42	2.32	2.05	2.26

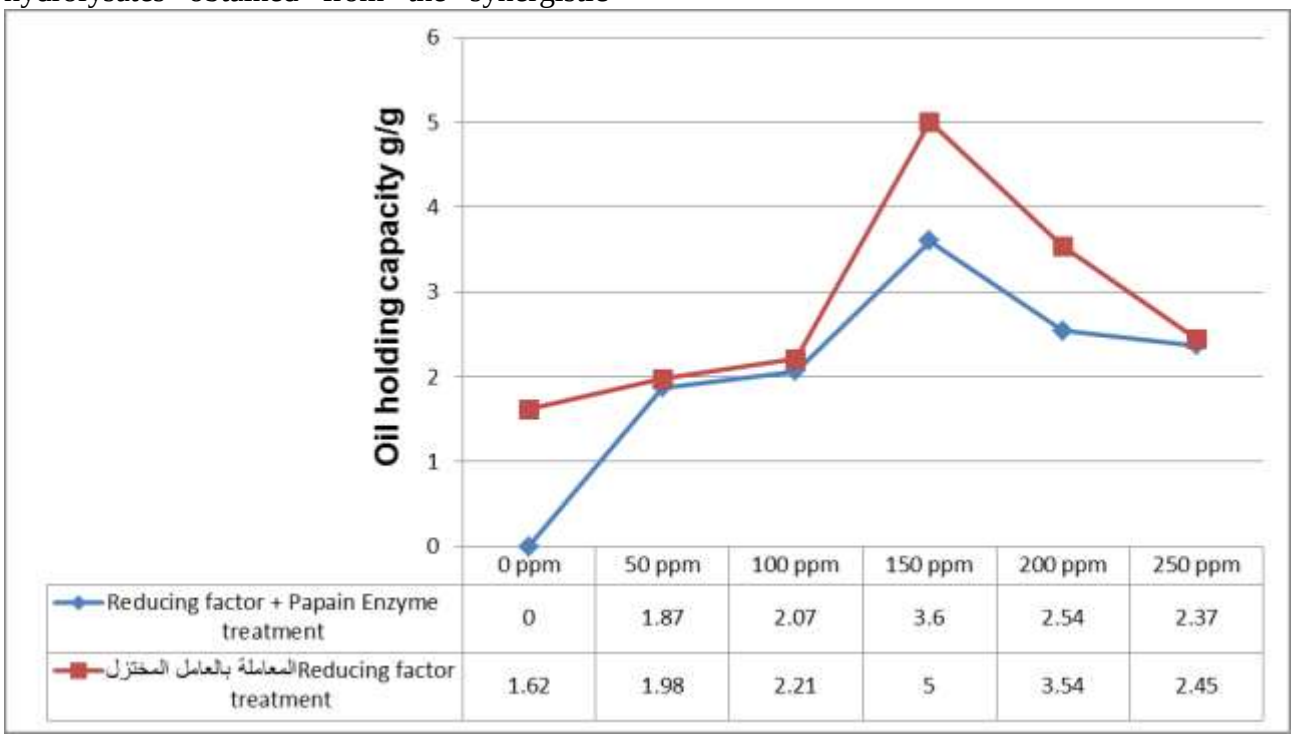
Figure 4. Water holding capacity for wheat protein treated with the reducing agent (L-Cysteine) at different concentrations and papain enzyme at a concentration of (2000 units / g of protein) at different pH values

The increase in solubility of the hydrolysates may be due to the function of the reducing agent alone or from the synergistic action of the reducing agent with the added enzyme. Additionally, enzyme or chemical agent would break more peptide bonds, which would contribute in reducing water holding capacity(34). Similarly, Wasswa *et al.* (31) found that the synergistic effect of the heat pretreatment and the enzymatic hydrolysis of gluten can liberate some peptides from wheat gluten which would improve the solubility and water holding of the treated proteins compared to the raw proteins. Elmalimadi *et al.* (8) noted that the synergistic action between the pre-heat treated and the enzymatic hydrolysis of gluten using Alcalase improved the solubility of gluten at pH values (2-12). The stability of the emulsion may also be improved, while the foam stability and the holding of oil and water did not improve. The antioxidants activity was improved significantly, as they used the gluten subjected to 75 ° C for (30) minutes.

Oil holding capacity(OHC)

Figure5 shows the effect of reducing agent (L-Cysteine) and enzymatic treatment (Papain) on the oil holding capacity of lyophilized gluten at pH values (12,7,4) for 4 hours. The results indicate that OHC of the gluten treated with reducing agent was positively proportional to (L-Cysteine) concentration up to 150 ppm ,

after that (200-250ppm) the OHC started to decline. The results also indicate that the treating gluten with the reducing agent and the papain synergistically improved the oil holding capacity at the concentrations of (50-150ppm) , the highest value (3.60%) was noticed at the concentration of (150ppm) in the presence of enzyme. While, at (200,250 ppm) the oil holding capacity began to decline. The hydrolysates obtained from the synergistic action of the reducing agent and the enzyme showed lower oil holding capacity as compared to reducing agent treated sample , and this may be due to the gluten degradation as a function of synergistic factors. Presence of the proteolytic enzyme causes breakdown of many peptide bonds, which impose negative effects on the oil and water holding capacity (31).

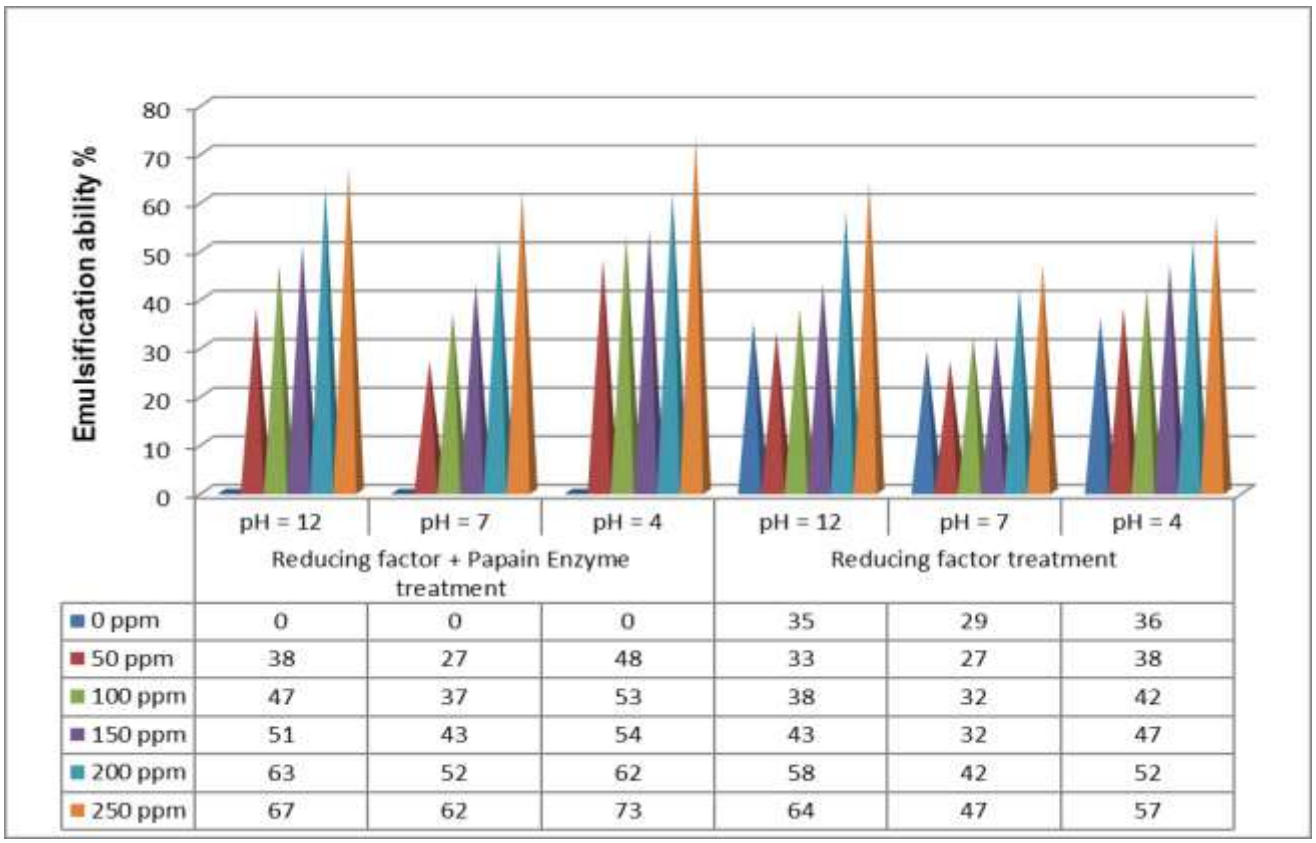


	0 ppm	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm
Reducing factor + Papain Enzyme treatment	0	1.87	2.07	3.6	2.54	2.37
Reducing factor المعاملة بالعامل المختزل treatment	1.62	1.98	2.21	5	3.54	2.45

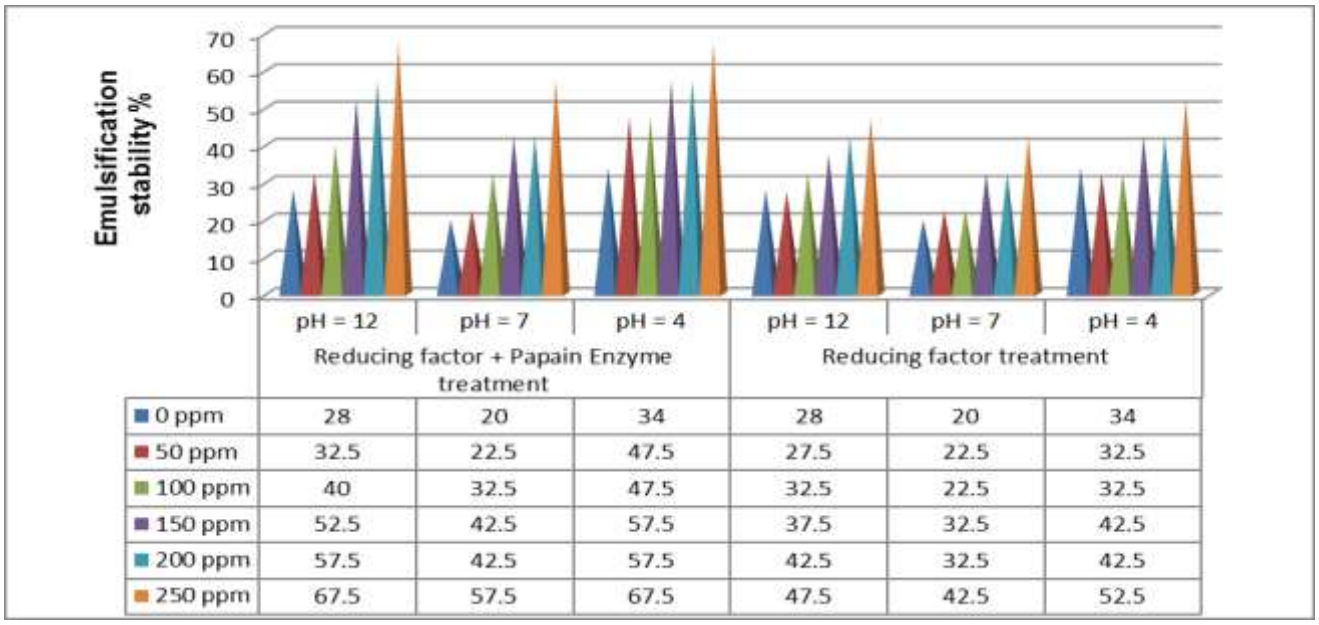
Figure 5. Oil holding capacity of lyophilized wheat gluten treated with (L-Cysteine) at different concentrations and papain (2000 units / g of protein) on different pH values

Emulsion Properties

Figure 6 indicates the percentage of emulsifying capacity and emulsion stability of gluten treated with a reducing agent (L-Cysteine) and reducing agent plus papain enzyme (2000 units / g of protein) synergistically at pH (12,7,4) for 4 hours. The results show that using the reducing agent showed a significant improvement in emulsification capacity at a concentration of (150 ppm) and pH values (4 and 12). At pH 7, using (200ppm) of(L-Cysteine) was effective to achieve a significant improvement in the raw gluten emulsion properteis. The results also indicate that the modified proteins using the reducing agent and the papain enzyme (2000 units / g of protein) recorded a significant improvement at the concentration of (50, 100, 150) and pH values (4, 7, 12), respectively. The results showed that the highest value of emulsification of chemically and enzymatically modified proteins were at pH. (12), while the lowest value recorded at pH (7). Likewise, the modified proteins using the same factors above showed a significant improvement in the stability of the emulsion at a concentration of (250ppm) and pH values (4, 7, 12). Eltayeb (10) found that the effect of pH values (9, 7.5, 6, 4.5, 3) on emulsification properties and emulsion stability of flour and field pistachio protein isolate were variable. The lowest emulsification capacity for flour and protein isolate was at pH 7.5, while the highest emulsification capacity was reached at pH 4.5). From Figure 6 the enzymatic treatment alone improved the emulsification capacity and emulsion stability of the proteins under study more than that with the reducing agent and the synergistic treatment using the reducing agent plus the enzyme.



	Reducing factor + Papain Enzyme treatment			Reducing factor treatment		
	pH = 12	pH = 7	pH = 4	pH = 12	pH = 7	pH = 4
0 ppm	0	0	0	35	29	36
50 ppm	38	27	48	33	27	38
100 ppm	47	37	53	38	32	42
150 ppm	51	43	54	43	32	47
200 ppm	63	52	62	58	42	52
250 ppm	67	62	73	64	47	57



	Reducing factor + Papain Enzyme treatment			Reducing factor treatment		
	pH = 12	pH = 7	pH = 4	pH = 12	pH = 7	pH = 4
0 ppm	28	20	34	28	20	34
50 ppm	32.5	22.5	47.5	27.5	22.5	32.5
100 ppm	40	32.5	47.5	32.5	22.5	32.5
150 ppm	52.5	42.5	57.5	37.5	32.5	42.5
200 ppm	57.5	42.5	57.5	42.5	32.5	42.5
250 ppm	67.5	57.5	67.5	47.5	42.5	52.5

Figure 6. Emulsification ability and emulsification stability of lyophilized wheat protein treated with the reducing agent (L-Cysteine) at different concentrations and papain (2000 units / g of protein) at different Ph

Foaming Ability: Table 1 shows the foaming ability of lyophilized wheat protein treated with a reducing agent (L-Cysteine) and reducing agent with presence of papain (2000 units / g of protein) synergistically at pH values (12,7,4) for (0, 15,30,45,60,) minutes. The results indicate that using the reducing agent only led to an improvement in its foam formation ability at all relevant concentrations, at all pH values. The highest value recorded at (150ppm) and pH (12), while the lowest value was at (50ppm) and pH (7). The results also indicate that the synergistic treatment of gluten with the reducing agent and papain enzyme improved the foaming ability at all applicable concentrations and at all pH levels more than that with L-Cysteine alone. The highest values were at the concentration of (150ppm) at t pH

of (12), and the lowest value were at the concentration (50ppm) at pH (7). The above results may be due to the solubility of the protein under study, so the treatment that recorded the highest solubility was recorded. highest foam formation at all pH levels and at all times.The results are consistent with (28) who found that the foaming capacity of wheat germ protein concentrate increases with pH increasing. This may be due to the increase in the electrical charge of the protein, which limits the hydrophobic interference and thus leads to an increase in the solubility and elasticity of the protein, leading to a faster spread of the protein on the surface separating water and air and increasing the foam capacity.

Table 1. Foaming ability of lyophilized wheat gluten treated with different concentration of reducing agent (L-Cysteine) and with papain enzyme (2000 units / g of protein) at different pH values

Reducing agent + Papain Enzyme						Reducing agent							
L.S.D	250 ppm	200 ppm	150 Ppm	100 ppm	50 ppm	250 ppm	200 Ppm	150 Ppm	100 Ppm	50 ppm	0 Ppm	Conc pH	Time
7.61 *	92	92	97	87	82	77	77	82	72	67	60	4	0
7.64 *	87	82	87	82	72	72	72	77	67	52	55	7	
7.96 *	92	92	97	92	87	77	82	82	77	72	66	12	
7.43 *	82	87	92	82	77	72	72	77	67	62	56	4	15
7.42 *	82	77	82	77	67	62	67	72	62	47	45	7	
7.65 *	87	87	92	87	82	72	77	77	72	67	60	12	
7.37 *	77	82	87	77	72	62	62	72	62	52	48	4	30
8.70 *	77	72	82	72	62	52	57	67	52	47	42	7	
7.50 *	82	82	87	82	77	67	72	72	67	62	56	12	
6.94 *	72	77	82	73	62	57	57	68	56	48	45	4	45
7.88 *	61	68	73	62	57	48	52	62	47	33	36	7	
7.55 *	77	78	76	72	67	63	67	68	61	52	48	12	
7.96 *	68	72	73	68	52	47	48	58	46	37	35	4	60
8.07 *	57	62	63	53	47	38	42	52	38	22	24	7	
8.49 *	72	73	68	62	57	53	58	57	53	42	38	12	
LSD	7.64*	7.56*	7.41*	7.05*	7.44*	8.12*	7.74*	6.98*	7.25*	8.61*		---	

Table (2) shows the stability of lyophilized wheat gluten foam treated with the reducing agent (L-Cysteine) and reducing agent plus papain (2000 units / g of protein) synergistically at pH (12, 7, 4) (60, 45, 30, 15, 0) min. The foam stability at pH (12) recorded the highest value while the lowest value was seen at pH (4), and the increase in foam stability may be due to the increase of protein solubility at high pH value ,which in turn leads to an increase in the amount of protein diffusion to the surface between water and air, which causes a decrease in the surface tension and improve the foam stability.

Table 2. Foaming stability for lyophilized wheat gluten treated with different concentrations of reducing agent (L-Cysteine) and with papain (2000 units / g of protein) at different pH

Reducing agent+ Papain Enzyme						Reducing agent							
L.S.D	250 ppm	200 ppm	150 ppm	100 ppm	50 ppm	250 ppm	200 ppm	150 Ppm	100 Ppm	50 ppm	0 Ppm	conce pH	Time
7.84 *	76	73	83	71	72	61	68	72	62	57	55	4	0
7.48 *	68	62	71	67	62	52	57	68	46	42	40	7	
7.42 *	83	77	87	82	77	67	72	77	67	62	59	12	
7.44 *	67	68	76	71	67	66	63	71	58	52	51	4	15
7.2 *	63	58	66	62	57	48	51	62	41	35	34	7	
7.55 *	78	73	82	76	71	63	68	76	62	57	55	12	
7.37 *	63	63	72	56	56	58	53	67	46	41	39	4	30
7.61 *	53	53	62	46	46	43	48	52	41	36	33	7	
7.50 *	73	73	77	66	61	63	63	71	52	46	41	12	
6.96 *	68	52	63	51	46	48	48	56	37	31	30	4	45
7.99 *	47	48	51	42	36	38	42	41	26	22	20	7	
6.15 *	63	68	67	61	56	58	52	61	41	38	34	12	
7.58 *	52	42	57	42	32	42	42	47	32	27	27	4	60
7.60 *	41	38	47	33	27	33	37	37	28	21	18	7	
6.44 *	57	58	62	52	46	47	42	57	37	32	30	12	
LSD	7.43 *	7.08 *	7.32 *	7.17 *	6.93 *	7.24*	6.55*	7.13 *	7.95*	7.41*	7.31*	---	

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